FINAL

Nov 3 1994

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DATA EVALUATION REPORT

Deoxy Avermectin

Study Type: Metabolism

Prepared for:

Health Effects Division
Office of Pesticide Programs
Environmental Protection Agency
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Arlington, VA 22202

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Work Assignment Number: 3-25
Clement Number: 85, 86
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DATA EVALUATION REPORT

STUDY TYPE: Metabolism - rats (85-1)

Tox. Chem. Number: New Chemical

P.C. CODE: 122806

MRID Number: 428515-23, 428515-24

TEST MATERIAL: [^{14}C]4"-Deoxy-4"-epimethylamino avermectin B1a benzoate

SYNONYMS: MAB1a benzoate, Deoxy Avermectin

LABORATORY REPORT NUMBER: ARM-5 (1 volume); ARM-6 (2 volumes)

SPONSOR: Agricultural Research and Development, Merck & Co., Inc., Hillsborough Road, Three Bridges, NJ 08887

TESTING FACILITY: Merck Research Laboratories, Animal & Exploratory Drug Metabolism, Hillsborough Road, Three Bridges, NJ 08887

TITLE OF REPORTS: (1) [^{14}C]4"-Deoxymethylamino Avermectin B1a: Determination of [^{14}C]CO_2 in Exhaled Air of Male and Female Rats after [^{14}C]4"-Deoxy-4"-epimethylamino Avermectin B1a (MAB1a) Benzoate Administration (A Preliminary Study); (2) The Tissue Distribution, Metabolism, and Excretion of [^{14}C]4"-Deoxy-4"-epimethylamino Avermectin B1a (MAB1a) Benzoate in Rats

AUTHOR: Mohammad Mushtaq

REPORTS ISSUED: June 29, 1993

CONCLUSION: The study demonstrated that radiolabeled MAB1a benzoate is rapidly absorbed, distributed, and excreted following oral and i.v. administration in rats. Total 7-day recoveries of the radioactivity were high for all groups (≈96-104% of the administered dose). The feces was the major route of excretion in oral and i.v. groups (≈94-103% of administered dose), while <1% of the administered dose was recovered in the urine at 7 days postdosing. Tissue distribution and bioaccumulation of MAB1a benzoate appeared to be minimal since radioactivity was <2% in tissues 7 days after oral and i.v. administration. The metabolism of MAB1a benzoate appears to involve primarily N-demethylation to AB1a. AB1a was the only metabolite detected in the feces while unmetabolized parent compound represented a large amount of the radioactivity in the feces.

Classification: Core-guideline

This study does satisfy the guideline requirement for a metabolism study (85-1) in rats.

Special Review Criteria (40 CFR 154.7): None

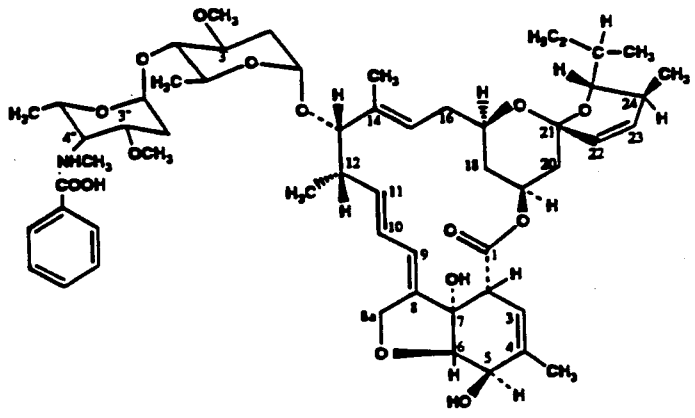
A. MATERIALS

1. Test compound:

Unlabeled MK-0244 (Lot number L-656,748-038W002) was used for the 14-day pretreatment period in the repeated-dosing experiment. [^{14}C]MAB1a benzoate (Lot number L-683,825-003E003) was used for all dosing groups. In the high-dose group, [^{3}H]MAB1a benzoate (Lot number L-683,825-001A006) was used to determine the stability of the tritium label at the 5-position in the MAB1a molecule.

	Unlabeled MK-0244	[^{14}C]MAB1a benzoate	[^{3}H]MAB1a benzoate
Description	white powder	Not reported	Not reported
Purity	95% (area percent) MAB1a benzoate; 5% MAB1b benzoate	≈94%	97%
Specific Activity	Not applicable	0.0288 mCi/mg	12265 mCi/mg
Position of radiolabel	Not applicable	3-,7-,11-, 13-,23-carbon	5-C hydrogen

The structure and radiolabel position of MAB1a benzoate are shown below:



2. Vehicle: propylene glycol/saline solution

3. Test animals:

Species: rats

Strain: Sprague-Dawley
Age and weight at study initiation: ≈5-weeks old; 211-266 g (males), 152-189 g (females)
Source: Charles River Breeding Laboratories, Inc., Wilmington, MA
Housing: individually in stainless-steel wire-mesh cages; Nalgene metabolism cages for housing animals catheterized in femoral artery
Environmental conditions:
Temperature: 72-76°F
Humidity: 20-70%
Air changes: Not reported
Photoperiod: 12 hour light/dark cycle
Acclimation period: ≈1 week

4. Preparation of dosing solutions:

The [^{14}C]MAB1a benzoate dosing solution administered to the low-dose oral and i.v. groups was prepared by mixing purified [^{14}C]MAB1a benzoate with MK-0244, and then diluting the mixture with methanol. The solution had a specific activity of 28.1 μCi/mg for males and 24.1 μCi/mg for females. The radiochemical purity was 97.7-98.1%.

The [^{14}C]MAB1a benzoate dosing solution administered to the repeated-dosing group was prepared by mixing [^{14}C]MAB1a benzoate with unlabeled MK-0244 in propylene glycol/saline solution. The solution was dried under nitrogen then reconstituted in methanol. The dosing solution had a specific activity of 17.7 μCi/mg and radiochemical purity of 91.8%.

The [^{3}H/^{14}C]MAB1a benzoate dosing solution administered to the high-dose group was prepared with [^{14}C]MAB1a benzoate, [^{3}H]MAB1a, and unlabeled MK-0244 in benzoic acid solution. The benzoic acid was used to convert [^{3}H]MAB1a into its benzoate salt. The specific activities were 34 μCi/mg for [^{3}H]MAB1a benzoate and 5.8 μCi/mg for [^{14}C]MAB1a benzoate. The radiochemical purities were 96.8% for [^{3}H]MAB1a benzoate and 94.9% for [^{14}C]MAB1a benzoate. The dosing solutions were essentially homogeneous as indicated by the mean dpm with standard deviation of only ±3.5% from aliquots taken from the top, middle, and bottom of the dosing samples, assayed before and after dose administration.

B. STUDY DESIGN

A total of 80 rats was used in the study. The study was designed to determine the absorption, distribution, metabolism, and excretion of labeled MAB1a benzoate after intravenous and oral administration to rats. The test groups and dose levels used in the study are shown in Table 2. The doses were selected based on an acute oral LD_{50} of 70 mg/kg for MK-0244 in rats[1]. The low dose of 0.5 mg/kg corresponded to a no-effect level, and the high dose of 20 mg/kg was selected in order to produce some toxic effects.

[1]Technical data sheet: MK-0244 Experimental Insecticide. Merck Sharp and Dohme Research Laboratories, Agricultural Research and Development, Hillsborough Road, Three Bridges, NJ 08887.

Group	Dose level (mg/kg)	Route of Administration	No. of Animals[a]		Time of Sacrifice
			Male	Female	
i.v. dose [^{14}C]	0.5	i.v.	12[b]	12[b]	1 or 7 days
Low-dose [^{14}C]	0.5	oral	12[b]	12[b]	7 days
Repeated-dose[c] [^{14}C]	0.5	oral	6	6	7 days
High-dose [^{3}H/^{14}C]	20	oral	6	6	7 days

[a]For each group, an additional male rat and female rat pretreated with unlabeled MK-0244 served as controls.
[b]Six of the 12 animals were catheterized in femoral artery one day prior to administration of radiolabeled dose.
[c]The animals received repeated oral doses of non-radiolabeled test material by gavage for 14 days. On day 15 of the study, the animals were administered labeled test material as a single oral dose.

C. TREATMENT OF ANIMALS AND ANALYSIS OF EXCRETA & TISSUES:

1. Oral and Intravenous Administration

Urine (including cage rinses) and feces were collected separately at 8 hours and 1, 2, 3, 4, 5, 6, and 7 days after administration. Urine samples and fecal homogenates were weighed and stored at ≈-10°F until processing and radioanalysis by liquid scintillation counting (LSC).

2. Tissue Distribution Study

Animals from all dose groups were sacrificed 7 days after administration, and the tissues were dissected out to analyze for radioactivity. In the low-dose oral and i.v. groups, 6 rats/sex also were sacrificed at 1 day postdosing. Tissue samples collected included the following: bone, brain, fat, gastrointestinal tract, heart, kidney, liver, lung, muscle, spinal cord, spleen, testes or uterus and ovaries, and residual carcass. Tissue samples were homogenized, if necessary, and then homogenates were analyzed for radioactivity by tissue combustion and/or LSC.

3. Identification of Metabolites

Fecal homogenates were pooled by dose, sex, and collection time. Homogenates were extracted with acetone and ethyl acetate and passed through a benzenesulfonyl-2 cartridge column (equilibrated with ethyl acetate), dried under nitrogen, and reconstituted in methanol. Metabolites were detected by using reversed-phase high-performance liquid chromatography (HPLC). The 1, 3, and 7 day postdosing samples from the high-dose males and females were used to isolate metabolites.

One metabolite was found that had a retention time similar to that of the ABla (4"-deoxy-4"-epiaminoavermectin Bla) standard. The standard-metabolite mixture was cochromatographed by Lichrosorb RP-18 column, Axxi-Chrom ODS column, or Axxi-Chrom silica column. The metabolite, ABla, was confirmed by ultraviolet, mass and NMR spectral analyses using the day-3 pooled fecal homogenates from the high-dose males.

5. Pharmacokinetic analysis

Blood was collected (from the catheters in the femoral arteries of the low-dose oral and i.v. groups) at 2, 4, 8, 12, 18, and 24 hours and 2, 3, 4, 5, 6, and 7 days postdosing. Plasma was removed, and the amount of radioactivity in plasma was determined by combustion followed by LSC. Blood collected at these sampling time points was used to determine the area under the curve (AUC), which was used to calculate the bioavailability and half-life of labeled MABla benzoate.

D. STATISTICS

Statistical analysis was performed on the data for total radioactive residues and percents of total dose in tissues and excreta. Averages and standard deviations were calculated.

E. QUALITY ASSURANCE

A signed and dated quality assurance statement was present.
A signed and dated GLP statement was present.

F. RESULTS

In a preliminary study (ARM-5), [^{14}C]MABla benzoate was administered by oral gavage to two male (0.38 mg/kg) and two female (0.45 mg/kg) rats. Expired $^{14}CO_2$ was collected in alkaline solutions at 0-24 and 24-48 hours postdosing. Results showed that the test material was not eliminated as $^{14}CO_2$ in rats.

1. Elimination and Recovery

As shown in Table 1, total recovery of ^{14}C radioactivity ranged from 96.23% to 104.18% of the administered dose at 7 days postdosing, with nearly all of the radioactivity eliminated in the feces (94.43-103.90% of the administered dose). The majority of the fecal radioactivity was recovered by 3 days postdosing. The recovery of radioactivity in the urine was minimal (0.06-0.34% of the administered dose at 7 days postdosing). In the high-dose group, the recovery pattern for ^{3}H-labeled radioactivity was similar to that for ^{14}C-labeled radioactivity. Because recoveries with the ^{3}H and ^{14}C labels were similar, the ^{3}H label at the 5-C position was considered stable.

2. Tissue Distribution

Total radioactivity recovered in the tissues and carcass was low (0.07-1.59% of the administered dose at 7 days postexposure, Table 1).

Tissue distribution was similar in both sexes and after all dosing regiments, with the highest tissue residues (in ppb equivalents) seen in the high-dose males. The highest levels were in the lungs (2033 ppb), followed by spleen (1281 ppb), gastrointestinal tract (753 ppb), testes (880 ppb), ovaries (872 ppb), kidneys (675 ppb), liver (578 ppb), fat (386 ppb), bone (228 ppb), heart (187 ppb), muscles (121 ppb), brain (13 ppb), and blood (15 ppb) (Table XVII, p. 90 of Report No. ARM-6).

3. Overall half-lives

Radioactivity in the plasma was highest at 4-18 hours postdosing, with most of the radioactivity eliminated by 24 hours postdosing. After oral administration, the half-life of MABla benzoate was 34.36 hours in males and 51.05 hours in females. After i.v. administration, the half-life was 28.64 hours in males and 40.66 hours in females. The bioavailability of MABla benzoate was determined to be 54.6% in males and 74.3% in females.

4. Metabolites

A single metabolite was detected in the feces following oral administration of MABla benzoate to rats. Reversed-phase HPLC analyses indicated that there was one fecal metabolite, ABla (N-demethylated product) (see Figure 1 for structure), which coeluted with the ABla standard. The metabolite was confirmed by nuclear magnetic resonance and MS. ABla in all dose groups represented 1-6% of the radioactivity in the HPLC peak of ABla on day 1 postdosing and increased to 13-20% on day 7 postdosing. Unmetabolized parent compound represented 45.77-56.22%, 66.95-75.82%, and 71.75-73.15% of the total radioactivity under the HPLC peak of MABla for the repeated-dose, low-dose, and high-dose groups, respectively (see attached Appendices 1-3). Polar peaks were detected only when the residue levels in the feces were low; however, they were not characterized due to the low radioactivity in these samples. ABla also was detected in the liver, kidney, muscle, and fat (5-15% of the tissue sample on day 1 postdosing and 4-23% on day 7 postdosing).

Major reaction: metabolic conversion of 4"-epimethylamino group to a 4"-epiaminogroup via demethylation

G. DISCUSSION

The study demonstrated that radiolabeled MABla benzoate is rapidly absorbed, distributed, and excreted following oral and i.v. administration in rats. Total 7-day recoveries of the radioactivity were high for all groups (≈96-104% of the administered dose). The feces was the major route of excretion in oral and i.v. groups (≈94-103% of administered dose), while <1% of the administered dose was recovered in the urine at 7 days postdosing. The half-life of MABla benzoate in plasma was longer in females (51.05 hours) than males (28.64 hours), and the study author reported that the bioavailability of MABla benzoate was higher in females (74.3%) than males (54.6%) after oral administration. Tissue distribution and bioaccumulation of MABla benzoate appeared to be minimal since radioactivity was <2% in tissues 7 days after oral and i.v. administration. The metabolism of MABla benzoate appears to

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involve primarily N-demethylation to ABla. Unmetabolized parent compound represented a large amount of the radioactivity in the feces and ABla was the only metabolite detected in the feces.

H. STUDY DEFICIENCIES

No major deficiencies were found in the review of the study.

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TABLE 1. Percent Recoveries of Orally or Intravenously Administered MABla Benzoate in Rats at 7 Days Postdosing

Dose Group[a]	Sex	Percentage of Administered Dose Recovered			
		Feces	Urine	Tissues	Total Recovery
Low-Dose Group					
[^{14}C] 0.5 mg/kg (single oral)	Male	102.93	0.12	0.44	103.48
	Female	103.90	0.06	0.22	104.18
Repeated-Dose Group[b]					
[^{14}C] 0.5 mg/kg	Male	102.19	0.25	0.81	103.26
	Female	103.35	0.14	0.25	103.74
High-Dose Group					
[^{14}C] 20 mg/kg	Male	94.43	0.34	1.46	96.23
	Female	94.86	0.29	1.24	96.38
[^{3}H] 20 mg/kg	Male	101.37	0.31	1.59	103.27
	Female	101.62	0.26	1.36	103.24
Intravenous-Dose Group					
[^{14}C] 0.5 mg/kg (intravenous)	Male	101.62	0.26	0.12	102.00
	Female	103.01	0.17	0.07	103.25

[a]Animals received the test material labeled with ^{14}C label. The high-dose group received [^{3}H/^{14}C] label.

[b]Animals were given 0.5 mg/kg/day unlabeled MK-0244 for 14 days and a single dose of 0.5 mg/kg [^{14}C]MABla benzoate on day 15.

Source: Extracted from Table XIV, p. 87 of the Study Report

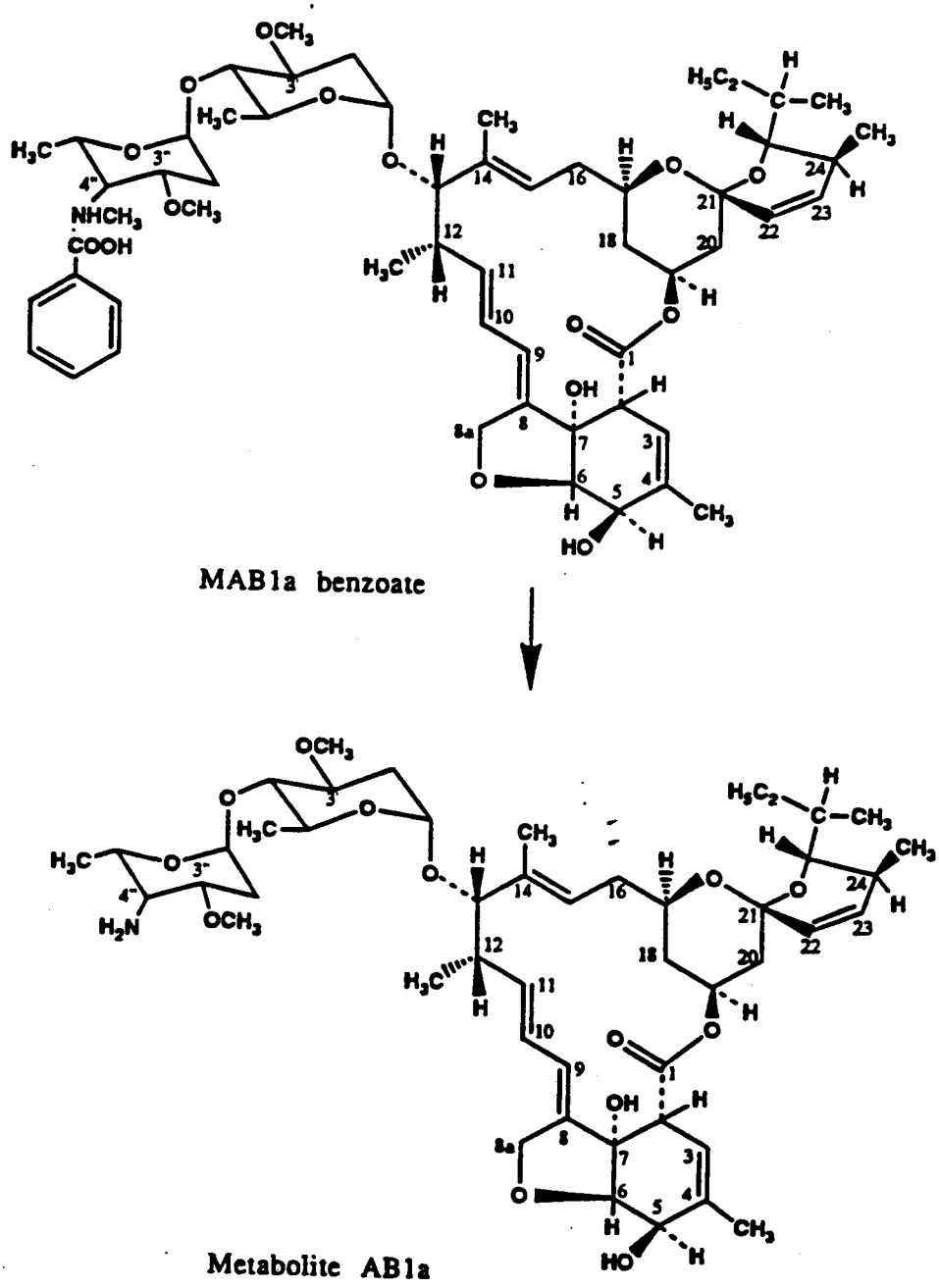

Figure 1. In vivo metabolism of MABla benzoate to its N-demethylated product, ABla, in rats.

Source: Study Report ARM-6, p. 131

Appendices 1-3
(Study Report No. ARM-6, pp. 357, 481, 560, and 561)

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AVERMECTIN

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